Cytoprotective Effects of Organosulfur Compounds against Methimazole-Induced Toxicity in Isolated Rat Hepatocytes

Reza Heidari[1,2,3], Hossein Babaei[1,2], Mohammad Ali Eghbal[1,2]*

[1] *Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.*

[2] *Pharmacology and toxicology department, School of pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.*

[3] *Students' Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.*

A R T I C L E I N F O

A B S T R A C T

Purpose: Methimazole is a drug widely used in hyperthyroidism. However, life-threatening hepatotoxicity has been associated with its clinical use. No protective agent has been found to be effective against methimazole-induced hepatotoxicity yet. Hence, the capacity of organosulfur compounds to protect rat hepatocytes against cytotoxic effects of methimazole and its proposed toxic metabolite, N-methylthiourea was evaluated. *Methods:* Hepatocytes were prepared by the method of collagenase enzyme perfusion via portal vein. Cells were treated with different concentrations of methimazole, N-methylthiourea, and organosulfur chemicals. Cell death, protein carbonylation, reactive oxygen species formation, lipid peroxidation, and mitochondrial depolarization were assessed as toxicity markers and the role of organosulfurs administration on them was investigated. *Results:* Methimazole caused a decrease in cellular glutathione content, mitochondrial membrane potential (ΔΨm) collapse, and protein carbonylation. In addition, an increase in reactive oxygen species (ROS) formation and lipid peroxidation was observed. Treating hepatocytes with N-methylthiourea caused a reduction in hepatocytes glutathione reservoirs and an elevation in carbonylated proteins, but no significant ROS formation, lipid peroxidation, or mitochondrial depolarization was observed. N-acetyl cysteine, allylmercaptan, and diallyldisulfide attenuated cell death and prevented ROS formation and lipid peroxidation caused by methimazole. Furthermore, organosulfur compounds diminished methimazole-induced mitochondrial damage and reduced the carbonylated proteins. In addition, these chemicals showed protective effects against cell death and protein carbonylation induced by methimazole metabolite. *Conclusion:* Organosulfur chemicals extend their protective effects against methimazole-induced toxicity by attenuating oxidative stress caused by this drug and preventing the adverse effects of methimazole and/or its metabolite (s) on subcellular components such as mitochondria.

Introduction

Methimazole is one of the most effective drugs in managing hyperthyroidism in humans.[1] However, serious adverse effects such as hepatotoxicity and agranulocytosis accompany its clinical use.[2,3] The mechanism(s) by which methimazole induces hepatotoxicity is not clearly understood yet and no protective agent has been found to be effective against its toxicity. In some investigations, it has been shown that reactive metabolites formed during methimazole metabolism could be the cause of cellular damage and toxicity. It was found that a kind of cytochrome P450 enzyme might be responsible for biotransformation of methimazole to its reactive metabolite that could be involved in cellular damage caused by this drug in olfactory mucosa.[4,5] N-methylthiourea is one of the methimazole metabolites which is generated by

cytochrome P450 enzymes and is suspected to be responsible for methimazole-induced hepatotoxicity.[6] N-methylthiourea is further metabolized to some reactive nucleophilic metabolites,[6] which are capable of binding to different cellular targets,[7] and causing cell dysfunction and toxicity. In another study, we have shown that methimazole cytotoxicity towards hepatocytes could be attributed to its reactive intermediates.[8]

Reactive metabolites interact with many cellular targets, especially proteins, and affect cell function. These events could result in toxicity and finally cell death. Glutathione is a thiol containing molecule that conjugate with electrophilic reactive intermediates of xenobiotics and prevent cellular damage.[9] In some experiments, it has been shown that glutathione had a

pivotal role in preventing methimazole-induced toxicity and this drug caused a severe hepatic injury in mice depleted of glutathione.[10] Depleting glutathione reservoirs had the same effects on the toxicity induced by methimazole in mice olfactory mucosa.[5] In another study, it was shown that methimazole inhibited some forms of CYP450 enzymes in the absence of glutathione, probably by its reactive metabolites.[11]

2-propen-1-thiol (Allyl mercaptan), and Diallyl disulfide, are small sulfur containing molecules derived from *Allium sativum* (garlic).[12] These compounds in contribution with other molecules are responsible for garlic odor and have been formed in breath after garlic ingestion.[12,13] It has been shown that organosulfurs had protective properties against xenobiotics-promoted cellular damage and oxidative stress in many cases such as carbon tetrachloride (CCl_4),[14] acetaminophen,[15,16] doxorubicin,[17,18] cyclophosphamide,[19] and aflatoxin B_1.[20] Another organosulfur chemical, N-acetylcysteine (NAC) has found its role in clinic and is used as a standard treatment in drugs-induced hepatotoxicity such as acetaminophen intoxication in humans.[21]

Because of their protective roles observed in previous investigations and their capability in scavenging reactive species,[22,23] this study attempted to evaluate the beneficial effects of organosulfur chemicals against methimazole-induced cellular injury in an *in vitro* model of isolated rat hepatocytes. Cell death, reactive oxygen species (ROS) formation, lipid peroxidation, protein carbonylation, and mitochondrial damage were considered as toxicity markers and the effects of organosulfur compounds on them were studied. Furthermore, the levels of cellular reduced and oxidized glutathione were measured to evaluate the ability of organosulfur compounds in preventing methimazole-induced hepatotoxicity.

Materials and Methods
Chemicals
Methimazole was purchased from Medisca pharmaceutique incorporation (Montreal, Canada). N-acetyl cysteine (NAC), 2-vinyl pyridine, Triethanolamine, Tris (hydroxymethyl) aminomethane, Oxidized glutathione (GSSG), and (4-(2-hydroxyethyl) 1-piperazine-ethanessulfonic acid (HEPES) were obtained from Acros (New Jersey, USA). Albumine bovine type was purchased from Roche diagnostic corporation (Indianapolis USA). 2-propen-1thiol (Allyl mercaptan), Diallyl disulfide (DADS), Rhodamine 123, 5,5′-dithio-bis(2-nitro-benzoicacid)(DTNB), 2,4-Dinitrophenyl hydrazine (DNPH), Guanidine, 2',7'Dichlorofluorescin diacetate, Glutathione reductase from baker's yeast, β-Nicotinamide adenine dinucleotide (NADPH), and Collagenase from clostridium histolyticum, were obtained from Sigma Aldrich (St. Louis, USA). Reduced glutathione (GSH), N-methylthiourea, Trichloro acetic acid (TCA), Ethyleneglycol-bis (ρ-aminoethylether)-N,N,N′,N′-tetra

acetic acid (EGTA), and Trypan blue were obtained from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) was obtained from SERVA (Heidenberg, New York). All salts used for preparing buffer solutions were of analytical grade and obtained from Merck (Darmstadt, Germany).

Hepatocyte preparation
Male Sprague–Dawley rats weighing 250–300 g were cared in plastic cages in an ambient temperature of 25±3 °C. Animals were fed a normal diet and water *ad libitum*. Collagenase perfusion method was used to isolate rat hepatocytes.[24] This technique is based on liver perfusion with collagenase after removal of calcium ion (Ca^{2+}) with a chelator (EGTA 0.5 mM). Liver was perfused with different buffer solutions through the portal vein. Collagenase containing buffer solution destructs liver interstitial tissue and cause hepatocyte to be easily isolated in next steps. Isolated hepatocytes (10 mL, 10^6 cells/mL) were incubated in Krebs-Henseleit buffer (pH 7.4) under an atmosphere of 95% O_2 and 5% CO_2 in 50 ml round bottom flasks which continuously rotating into a 37 ℃ water bath.

Any of chemicals used for evaluating their protective effects, caused no significant toxicity toward hepatocytes as compared to the control cells when administered alone in given concentrations. The animals were handled and used, according the ethical guidelines of Tabriz University of Medical Sciences, Tabriz, Iran.

Cell viability
Trypan blue dye exclusion staining was used to assess the percentage of death cells.[25] Hepatocytes viability was determined at different time intervals to evaluate the effect of methimazole and N-methylthiourea on cell viability, determining LC_{50} (lethal concentration 50%) dose of the drugs and testing the protective effects of organosulfur compounds against cell death induced by methimazole or N-methylthiourea. Hepatocytes were at least 85% viable before their use.

Reactive oxygen species (ROS) formation
To control the extent of ROS formed during methimazole metabolism, 2, 7-dichlorofluorescein diacetate (1.6 μM) was added to the hepatocyte incubate. DCFH-DA became hydrolyzed to non-fluorescent dichlorofluorescein (DCFH) in hepatocytes. Dichlorofluorescin then reacted with reactive oxygen species to form the highly fluorescent dichlorofluorescein. 1mL (10^6 cells) of hepatocytes was taken and the fluorescence intensity was measured using a Jasco® FP-750 spectrofluorometer with excitation and emission wavelengths of 500 and 520 nm, respectively.[26]

Lipid peroxidation Measurement
Hepatocyte lipid peroxidation was determined by measuring the amount of thiobarbituric acid reactive

substances (TBARS) formed during the decomposition of lipid hydroperoxides. After treating 1mL aliquots of hepatocyte suspension (10^6 cells/mL) with trichloroacetic acid (70% w/v), the supernatant was boiled with thiobarbituric acid (0.8% w/v) for 20 minutes. The absorbance of appeared color was determined using an Ultrospec® 2000 UV spectrophotometer at 532 nm.[27]

Protein carbonylation assay

Total protein-bound carbonyl content was measured by derivatizing the carbonyl adducts with DNPH at 30, 90, and 180 minutes. Briefly an aliquot of the suspension of cells (0.5 mL, 0.5×10^6 cells) was added to 0.5 mL of 0.1% DNPH (w/v) in 2.0 N HCl and allowed to incubate for 1 hour at room temperature. This reaction was stopped and total cellular protein precipitated by adding 1.0 mL of 20% TCA (w/v). Cellular protein was rapidly pelleted by centrifugation at 10,000 rpm, and the supernatant was discarded. Excess unincorporated DNPH was extracted three times using 0.5 mL of ethanol: ethyl acetate (1:1) solution each time. Following the extraction, the recovered cellular protein was solubilized in 1 mL of Tris-buffered 8.0 M guanidine–HCl, pH 7.2. The resulting solubilized hydrazones were measured at 366–370 nm. The concentration of 2,4-DNPH derivatized protein carbonyls was determined using the extinction coefficient of 22,000 M^{-1} cm^{-1}.[28]

Mitochondrial membrane potential

Mitochondrial membrane potential was assessed as an indicator of toxicity induced by methimazole or N-methylthiourea. The fluorescent dye, rhodamine 123 was used as a probe to evaluate the mitochondrial membrane potential in rat hepatocytes. Samples (1 mL) were taken from the cell suspension at scheduled time points, and centrifuged at 1000 rpm for 1 minute. The cell pellet was then resuspended in 2 mL of fresh incubation medium containing 1.5 µM rhodamine 123 and gently shaked in a 37 ºC thermostatic water bath for 10 minutes. Hepatocytes were separated by centrifugation (3000 rpm for one minute) and the amount of rhodamine 123 appearing in the incubation medium was measured fluorimeterically at 490 nm excitation and 520 nm emission wavelengths using a Jasco® FP-750 spectrofluorometer.[29]

Determination of Hepatocytes GSH/GSSG content

Hepatocytes reduced and oxidized glutathione (GSH and GSSG) content was determined using enzymatic recycling method.[30] For determination of GSH, a 1 ml aliquot of the cell suspension (10^6 cells) was taken and 2 ml of 5% TCA was added and centrifuged. Then 0.5 ml of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) were added. The absorbance of developed color was determined at 412 nm using an Ultrospec® 2000 spectrophotometer. To assess the hepatocytes' GSSG level, cellular GSH content was covalently bonded to 2-vinylpyridine at first. Then the excess of 2-vinylpyridine was neutralized with thriethanolamine and GSSG was reduced to GSH using glutathione reductase enzyme and NADPH. The amount of GSH formed was measured as described for GSH using Ellman reagent (0.0198% DTNB in 1% sodium citrate).[30]

Statistical analysis

Results are given as the Means±SE for at least three independent experiments. Statistical analysis was performed by a one-way analysis of variance (ANOVA) followed by a Tukey's *post hoc* test (SPSS software; version 16.0). A $P < 0.05$ was considered as significant difference.

Results

Trypan blue exclusion test was used to determine the ability of hepatocytes to maintain their viability with different concentrations of methimazole or N-methylthiourea, alone or in combination with organosulfur compounds. It was previously found that methimazole caused cell death in a concentration dependent manner.[8] The concentration in which the drug caused 50 % loss in hepatocytes viability (LC_{50}) was found to be 10 mM (Table 1).[8] The LC_{50} for the methimazole metabolite, N-methylthiourea was 1 mM (Table 1).[8] To determine the effects of organosulfur compounds on cell death induced by methimazole and/or N-methylthiourea, Diallyl disulfide (DADS), Allyl mercaptan, and N-acetyl cysteine were added to incubation medium. For this purpose, the LC_{50} of methimazole (10 mM) and N-methylthiourea (1 mM) were selected.

It was found that NAC and the other two organosulfur agents, Allyl mercaptan, and/or DADS decreased cell death induced by methimazole or N-methylthiourea (Table 1) ($P<0.05$). In another study on methimazole cytotoxicity, we found that depleting glutathione reservoirs by using 1-bromoheptane[31] had a deleterious effect on methimazole and/or N-methylthiourea-induced cytotoxicity.[8] Only 5 µM methimazole was needed to cause 50% cell death in glutathione depleted cells (data not shown) and all glutathione depleted hepatocytes were dead when 1 mM of N-methylthiourea was added to the cellular media (data not given).[8] These findings suggest the pivotal role of glutathione in preventing methimazole-induced cytotoxicity. N-acetyl cysteine effectively prevented cell death in glutathione-depleted hepatocytes (data not shown).[8]

Since glutathione depletion had a dramatic effect on cell death induced by methimazole and its metabolite, cellular glutathione content was measured to further investigate if methimazole or N-methylhiourea toxicity is related to GSH reduction in hepatocytes. The results showed that hepatocytes glutathione reservoirs were decreased significantly ($P<0.01$) when cells were

treated with methimazole and/or N-methylthiourea (Figure 1).[8] As previously showed in another study on methimazole,[8] this might indicate the importance of this thiol containing molecule to detoxify methimazole metabolites and preventing cellular damage induced by this drug. The presence of DADS in cellular media prevented glutathione (GSH) depletion induced by methimazole or its metabolite (P<0.05) (Figure 1). The amount of oxidized glutathione (GSSG) was elevated in hepatocytes treated with methimazole (P<0.01) (Figure 2), but there was no significant difference in

GSSG content between N-methylthiourea-treated cells and control group (Figure 2). Addition of 100 μM of DADS to cellular medium reduced (P<0.0.05) the amount of GSSG formed during methimazole administration (Figure 2). Elevation in oxidized glutathione in cells during chemical exposure is an indicator of the reactive oxygen species formation and occurrence of oxidative stress during toxic insult. Hence, the amount of ROS formation was evaluated to assess if methimazole or N-methylthiourea caused oxidative stress in isolated rat hepatocytes.[8]

Table 1. Methimazole and N-methylthiourea cytotoxicity in freshly-isolated rat hepatocytes.

Incubate		Cytotoxicity (% Trypan blue uptake)		
	Incubation time (min):	60	120	180
Control (only hepatocytes)		20±2	25±2	29±2
+ Methimazole 10 mM		43±2 [a]	55±3 [a]	68±4 [a]
+ N-acetyl cysteine 200 μM		23±3 [b]	32±2 [b]	35±3 [b]
+ Diallyldisulfide 100 μM		28±2 [b]	39±3 [b]	46±2 [b]
+ Allylmercaptan 200 μM		24±1 [b]	33±2 [b]	43±2 [b]
+ N-methyl thiourea 500 μM		29±4	38±5	54±3 [a]
+ N-methyl thiourea 1 mM		39±2 [a]	54±1 [a]	65±3 [a]
+ N-acetyl cysteine 200 μM		24±1 [c]	28±2 [c]	33±2 [c]
+ Diallyldisulfide 100 μM		28±2 [c]	39±3 [c]	58±1
+ Allylmercaptan 200 μM		23±1 [c]	40±2 [c]	44±3 [c]
+ N-methyl thiourea 2 mM		48±3 [a]	59±4 [a]	75±7 [a]

Isolated rat hepatocytes (10^6 cells/mL) were incubated at 37 °C in rotating round bottom flasks with 95 % O_2 and 5 % CO_2 in Krebs-Henseleit buffer (pH 7.4).
The results shown represent the Mean± SE for three independent experiments.
[a] Significantly different from control group (P < 0.05).
[b] Significant as compared to methimazole-treated group (P<0.05).
[c] Significantly different from N-methylthiourea treated group (P<0.05).
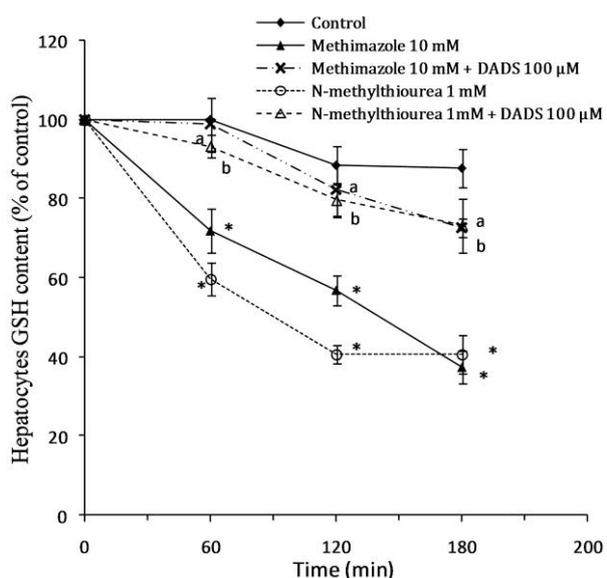

Figure 1. The effect of methimazole on hepatocytes GSH content. DADS: Diallyldisulfide.
Given data represent Means±SE for at least three separate experiments.
* Significantly different from control group (P<0.01).
[a] Significantly different from methimazole-treated group (P<0.05).
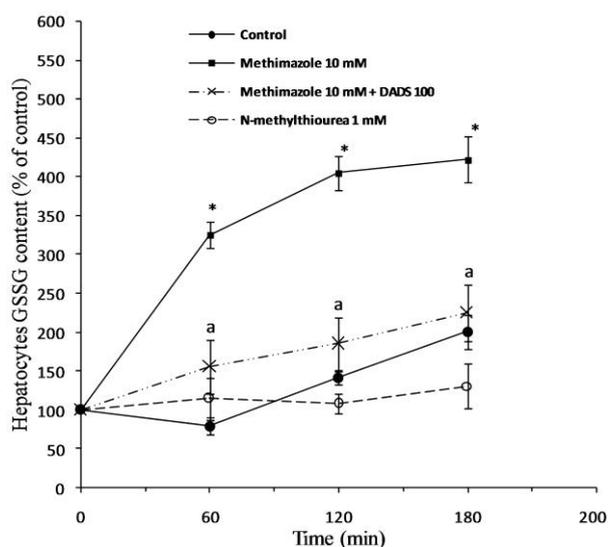

Figure 2. The level of oxidized glutathione (GSSG) formed after methimazole and N-methylthiourea. DADS: diallyldisulfide.
* Significantly different from control group (P<0.01).
[a] Significantly different from methimazole-treated group (P<0.05).

Treating rat hepatocytes with methimazole caused a significant elevation in reactive oxygen species formation (P<0.001) (Figure 3).[8] N-methylthiourea-treated hepatocytes showed no difference in ROS formation as compared to the control group (Figure 3).[8] Administration of organosulfur chemicals decreased ROS formation (P<0.05) induced by methimazole (Figure 3).
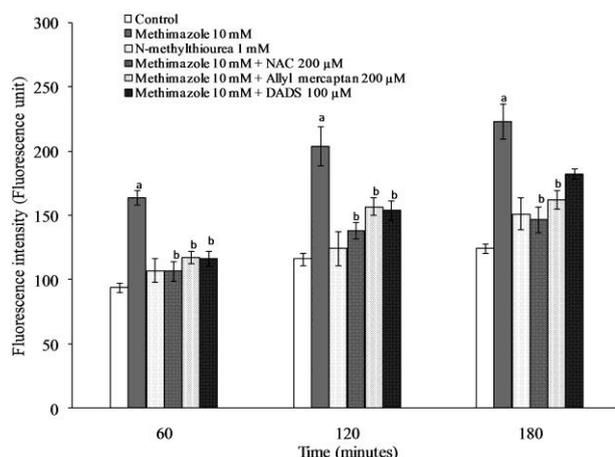

Figure 3. Methimazole-induced ROS formation in rat hepatocytes and the role of organosulfur compounds. DADS: Diallyl disulfide. NAC: N-acetylcysteine.
Data represent Mean±SE for three experiments.
[a] Significantly different from control group (P<0.001).
[b] Significantly different from methimazole-treated group (P<0.05).

Lipid peroxidation is a consequence of ROS formation and oxidative stress in biological systems.[32] It was found that a remarkable amount of thiobarbituric acid reactive substances (TBARS) was formed in methimazole-treated rat hepatocytes (P<0.001) as compared to the control group (Figure 4). This indicated the lipid peroxidation induced by cytotoxic concentrations of the drug. N-methylthiourea did not cause lipid peroxidation in rat hepatocytes.[8] Co-administration of NAC,[8] and/or other organosulfur chemicals considerably prevented (P<0.01) lipid peroxidation caused by methimazole (Figure 4).

The formation of carbonyl compounds is the most general and widely used marker of protein oxidation both *in vitro* and *in vivo*.[33] It was found that; protein carbonylation induced by methimazole was significantly higher (P<0.001) than the control level (Figure 5). Co-administration of organosulfur compounds reduced the level of protein carbonylation in methimazole-treated cells. The methimazole metabolite, N-methylthiourea caused an increase in carbonylated proteins (P<0.001) (Figure 5), which was significantly diminished (P<0.01) when organosulfur chemicals were added to hepatocytes incubation medium (Figure 5).

The changes in mitochondrial membrane potential (ΔΨ) as an important parameter of mitochondrial function were assessed previously to investigate if

cellular mitochondria are a target for methimazole and/or its metabolite.[8] The LC_{50} dose of methimazole (10 mM) caused mitochondrial depolarization (P<0.05) as measured with rhodamine 123 test (Figure 6).[8] This indicates that mitochondria could be a target for methimazole or its reactive metabolite(s) to cause hepatocyte damage and consequently cell death. However, N-methylthiourea did not cause any significant reduction in mitochondrial membrane potential (Figure 6).[8] This finding suggested that other methimazole metabolite(s) rather than N-methylthiourea might be responsible for the adverse effect of methimazole on mitochondria.[8] Organosulfur compounds were administered with methimazole to investigate if they could alleviate mitochondrial injury induced by this drug. It was found that NAC, Allyl mercaptan, and DADS effectively prevented mitochondrial depolarization (P<0.05) caused by methimazole (Figure 6).
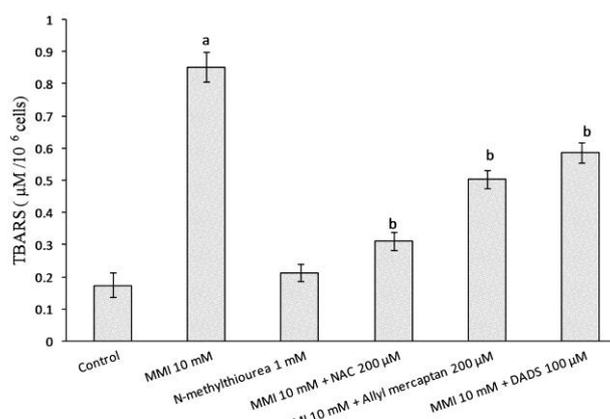

Figure 4. Methimazole-induced lipid peroxidation and the effect of organosulfur compounds. MMI: Methimazole (Methyl Mercapto Imidazole)
Data are shown as Mean±SE for three experiments as measured after 120 minutes.
[a] Significant as compared to control (P<0.001).
[b] Significant as compared to methimazole-treated group (P<0.01).

Discussion

Methimazole alone caused cell death in a concentration dependent manner. Methimazole-induced cytotoxicity was accompanied with ROS formation, lipid peroxidation, mitochondrial depolarization, and protein carbonylation. Furthermore, reduction in cellular glutathione (GSH) reservoirs, and increased hepatocytes' oxidized glutathione (GSSG) content was observed when hepatocytes were treated with methimazole. The suspected methimazole toxic metabolite, N-methylthiourea caused cell death concentration-dependently and reduced cellular glutathione content. In addition, a significant amount of carbonylated proteins was formed in N-methylthiourea treated hepatocytes, but no ROS formation, lipid peroxidation or changes in mitochondrial membrane potential were observed. Co-administration of organosulfur compounds diminished cell death induced

by methimazole or N-methylthiourea and reduced the consequences of methimazole induced toxicity such as ROS formation, lipid peroxidation, and mitochondrial damage.
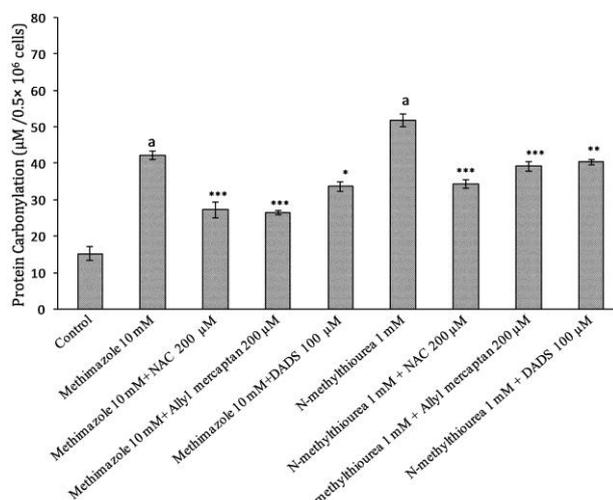

Figure 5. Protein carbonylation caused by methimazole and N-methylthiourea and the role of organosulfur compounds. DADS: Diallyl disulfide; NAC: N-acetyl cysteine.
Data are given as Mean±SE for three independent experiments as measured after 30 minutes of incubation.
[a] Significantly different from control (P<0.001).
[*] Asterisks indicate significant differences as compared to drug-treated groups (*P<0.05, **P<0.01 and ***P<0.001).
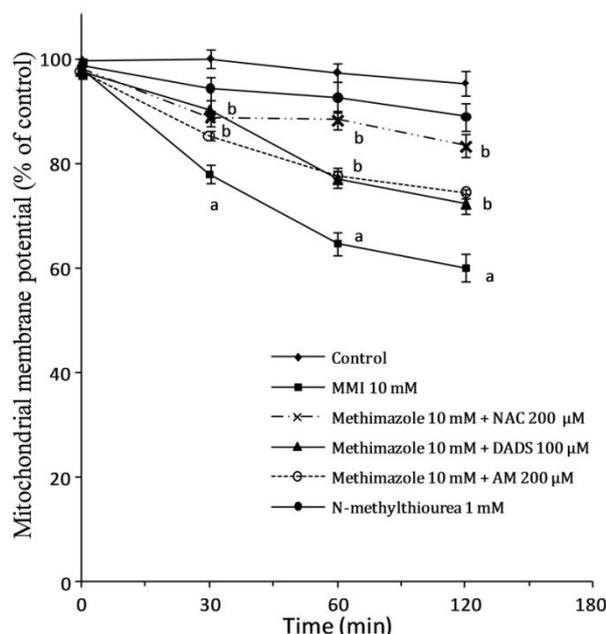

Figure 6. Methimazole-induced mitochondrial depolarization and the effects of organosulfur agents.
Data represent Mean±SE for three separate experiments.
[a] Different from control group (P<0.05).
[b] Significantly different from methimazole-treated group (P<0.05).

Reduction in glutathione reservoirs in hepatocytes treated with methimazole or N-methylthiourea,[8] indicates that GSH has a critical role in detoxifying methimazole metabolites and preventing cellular

damage. In previous studies it has been shown that glutathione depletion has deleterious effects on methimazole-induced toxicity in olfactory mucosa,[5] or mice liver.[10] Our results in isolated rat hepatocytes are in accordance with previous studies in this field.[8] Furthermore, our findings showed that methimazole caused an increase in ROS formation, lipid peroxidation, and reduction in hepatocytes mitochondrial membrane potential.[8] We found that the amount of oxidized glutathione (GSSG) was significantly raised in methimazole-treated cells, which indicates the occurrence of oxidative stress. Organosulfur chemicals such as diallyl disulfide significantly prevented glutathione reduction caused by methimazole or N-methylthiourea and reduced the GSSG formation during methimazole toxicity (Figure 2). This might indicate the importance of organosulfur chemicals in alleviating oxidative stress and scavenging reactive specie to prevent the consumption of glutathione as one of the main cellular defense mechanisms against toxic insults.

N-methylthiourea as the suspected toxic metabolite for methimazole[6] did not caused ROS formation, lipid peroxidation, or mitochondrial depolarization.[8] These finding suggest that other methimazole metabolite(s) rather than N-methylthiourea are responsible for observed adverse effects of methimazole in hepatocytes.[8] However, it has been shown that N-methylthiourea is further metabolized through flavin containing monooxygenase (FMO) enzyme to produce sulfenic acids.[6] Sulfenic acid species are reactive chemicals that covalently bind to nucleophilic sites.[7] Conjugating of sulfenic acids with glutathione could be a pathways for detoxifying them since N-methylthiourea caused reduction in cellular glutathione content and showed a sever toxicity profile in glutathione depleted cells. Binding of those reactive species to different targets such as proteins may cause cellular damage and toxicity. We found that N-methylthiourea caused an elevation in cellular carbonylated proteins level, which could be attributed to the cytotoxicity induced by N-methyl thiourea, and it is after head toxic metabolites. Since organosulfur agents significantly reduced protein carbonylation induced by N-methylthiourea (Figure 5), the protective effects of organosulfurs might be attributed to their effects on cellular protein damages caused by methimazole metabolite. In another investigation, we found that by administrating N,N-dimethylaniline as an FMO enzyme inhibitor,[34] the toxicity of N-methylthiourea in isolated hepatocytes was significantly reduced (Data not shown).[8]

The insignificant effect of N-methylthiourea on hepatocytes GSSG content supported the absence of oxidative stress during N-methylthiourea induced toxicity. This finding suggested that the reduction in hepatocytes glutathione (GSH) after treating cells with N-methylthiourea was due to conjugation of reactive metabolites with glutathione.[8]

The effect of methimazole and its metabolite on cell organelles such as mitochondria gives us an insight into the mechanisms by which this drug caused cytotoxicity toward isolated hepatocytes. Mitochondrial depolarization is accompanied by energy crisis and releasing of apoptotic signaling molecules, which could finally encounter cell death.[35] The insignificant effect of N-methylthiourea on mitochondrial membrane potential may indicate that the deleterious effect of methimazole on this organelle might be due to the other methimazole metabolite(s) rather than N-methylthiourea.[8] Administration of organosulfur chemicals might provide protection against methimazole-induced mitochondrial damage through their activity in attenuating oxidative stress and scavenging reactive species.

The use of glutathione-like thiol containing molecules such as allylmercaptan or other organosulfur compounds seems a reasonable choice to prevent methimazole-induced toxicity because of their non-enzymatic activities.[36] These compounds could scavenge reactive species[37,38] and chelating metal ions, which might have a role in preventing oxidative stress and lipid peroxidation.[39] The protective effects of organosulfur chemicals against methimazole and N-methylthiourea could be due to their capability in scavenging reactive metabolites. Furthermore, some organosulfur agents such as NAC might act as a glyoxal tarp and hence prevent methimazole-induced cytotoxicity.[8]

It seems that other methimazole metabolite(s) contributed with N-methylthiourea to induce cytotoxicity and the major part of organosulfurs protective effects in methimazole-induced toxicity might be due to their action against those metabolite(s). Further investigations including mass spectrometric analysis of conjugate formation is needed to elucidate subject.

The beneficial effect of organosulfur compounds against methimazole-induced toxicity proposes these agents as the subject of further studies for preventing different xenobiotics-induced liver damages, especially those accompanied with oxidative stress.

Conclusion

This study suggests that organosulfur chemicals extend their protective effects against methimazole-induced toxicity by attenuating oxidative stress caused by this drug. Furthermore, organosulfurs prevented the adverse effects of methimazole and its metabolite(s) on subcellular components such as mitochondria, which might has a role in attenuating the cytotoxicity induced by methimazole or its metabolite.

Acknowledgments

Conflict of Interest

There is no conflict of interest in this study.

References

1. Franklyn JA. Thyroid gland: Antithyroid therapy--best choice of drug and dose. *Nat Rev Endocrinol* 2009;5(11):592-4.
2. Garcia D, Peon N, Torres F, Hip-Avagnina MI, Martinez A, di Crocce ME. Four cases of thiamazole-induced agranulocytosis. *Farm Hosp* 2008;32(3):183-5.
3. Livadas S, Xyrafis X, Economou F, Boutzios G, Christou M, Zerva A, et al. Liver failure due to antithyroid drugs: Report of a case and literature review. *Endocrine* 2010;38(1):24-8.
4. Brittebo EB. Metabolism-dependent toxicity of methimazole in the olfactory nasal mucosa. *Pharmacol Toxicol* 1995;76(1):76-9.
5. Bergström U, Giovanetti A, Piras E, Brittebo EB. Methimazole-induced damage in the olfactory mucosa: effects on ultrastructure and glutathione levels. *Toxicol Pathol* 2003;31(4):379-87.
6. Mizutani T, Yoshida K, Murakami M, Shirai M, Kawazoe S. Evidence for the involvement of N-methylthiourea, a ring cleavage metabolite, in the hepatotoxicity of methimazole in glutathione-depleted mice: structure-toxicity and metabolic studies. *Chem Res Toxicol* 2000;13(3):170-6.
7. Mansuy D, Dansette PM. Sulfenic acids as reactive intermediates in xenobiotic metabolism. *Arch Biochem Biophys* 2011;507(1):174-85.
8. Heidari R, Babaei H, Eghbal MA. Mechanisms of methimazole cytotoxicity in isolated rat hepatocytes. *Drug Chem Txicol* 2012: doi: 10.3109/01480545.2012.749272
9. Forman HJ, Zhang H, Rinna A. Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Mol Aspects Med* 2009;30(1-2):1-12.
10. Mizutani T, Murakami M, Shirai M, Tanaka M, Nakanishi K. Metabolism-dependent hepatotoxicity of methimazole in mice depleted of glutathione. *J Appl Toxicol* 1999;19(3):193-8.
11. Kedderis GL, Rickert DE. Loss of rat liver microsomal cytochrome P-450 during methimazole metabolism. Role of flavin-containing monooxygenase. *Drug Metab Dispos* 1985;13(1):58-61.
12. Block E. The chemistry of garlic and onions. *Sci Am* 1985;252(3):114-9.
13. Rosen RT, Hiserodt RD, Fukuda EK, Ruiz RJ, Zhou Z, Lech J, et al. Determination of allicin, S-allylcysteine and volatile metabolites of garlic in breath, plasma or

simulated gastric fluids. *J Nutr* 2001;131(3s):968S-71S.

14. Fanelli SL, Castro GD, de Toranzo EG, Castro JA. Mechanisms of the preventive properties of some garlic components in the carbon tetrachloride-promoted oxidative stress. Diallyl sulfide; diallyl disulfide; allyl mercaptan and allyl methyl sulfide. *Res Commun Mol Pathol Pharmacol* 1998;102(2):163-74.

15. Sumioka I, Matsura T, Yamada K. Therapeutic effect of S-allylmercaptocysteine on acetaminophen-induced liver injury in mice. *Eur J Pharmacol* 2001;433(2-3):177-85.

16. Anoush M, Eghbal MA, Fathiazad F, Hamzeiy H, Kouzehkonani NS. The Protective Effect of Garlic Extract against Acetaminophen-Induced Loss of Mitochondrial Membrane Potential in Freshly Isolated Rat Hepatocytes. *Iran J Pharm Sci* 2009;5(3):141-50.

17. Mostafa MG, Mima T, Ohnishi ST, Mori K. S-allylcysteine ameliorates doxorubicin toxicity in the heart and liver in mice. *Planta Med* 2000;66(2):148-51.

18. Dwivedi C, John LM, Schmidt DS, Engineer FN. Effects of oil-soluble organosulfur compounds from garlic on doxorubicin-induced lipid peroxidation. *Anticancer Drugs* 1998;9(3):291-4.

19. Manesh C, Kuttan G. Alleviation of cyclophosphamide-induced urotoxicity by naturally occurring sulphur compounds. *J Exp Clin Cancer Res* 2002;21(4):509-17.

20. Sheen LY, Wu CC, Lii CK, Tsai SJ. Effect of diallyl sulfide and diallyl disulfide, the active principles of garlic, on the aflatoxin B(1)-induced DNA damage in primary rat hepatocytes. *Toxicol Lett* 2001;122(1):45-52.

21. Heard KJ. Acetylcysteine for Acetaminophen Poisoning. *N Engl J Med* 2008;359(3):285-92.

22. Zafarullah M, Li WQ, Sylvester J, Ahmad M. Molecular mechanisms of N-acetylcysteine actions. *Cell Mol Life Sci* 2003;60(1):6-20.

23. Kim JM, Chang HJ, Kim WK, Chang N, Chun HS. Structure-activity relationship of neuroprotective and reactive oxygen species scavenging activities for allium organosulfur compounds. *J Agric Food Chem* 2006;54(18):6547-53.

24. Heidari R, Babaei H, Eghbal MA. Ameliorative Effects of Taurine Against Methimazole-Induced Cytotoxicity in Isolated Rat Hepatocytes. *Sci Pharm* 2012;80:987-99.

25. Truong DH, Eghbal MA, Hindmarsh W, Roth SH, O'Brien PJ. Molecular Mechanisms of Hydrogen Sulfide Toxicity. *Drug Metab Rev* 2006;38(4):733-44.

26. Pourahmad J, Mortada Y, Eskandari MR, Shahraki J. Involvement of Lysosomal Labilisation and Lysosomal/mitochondrial Cross-Talk in Diclofenac

27. Niknahad H, O'Brien PJ. Mechanism of sulfite cytotoxicity in isolated rat hepatocytes. *Chem Biol Interact* 2008;174(3):147-54.

28. Tafazoli S, O'Brien PJ. Amodiaquine-induced oxidative stress in a hepatocyte inflammation model. *Toxicology* 2009;256(1-2):101-9.

29. Eghbal MA, Pennefather PS, O'Brien PJ. H2S cytotoxicity mechanism involves reactive oxygen species formation and mitochondrial depolarisation. *Toxicology* 2004;203(1-3):69-76.

30. Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat Protoc* 2006;1(6):3159-65.

31. Khan S, O'Brien PJ. 1-bromoalkanes as new potent nontoxic glutathione depletors in isolated rat hepatocytes. *Biochem Biophys Res Commun* 1991;179(1):436-41.

32. Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Ann Bot* 2003;91 Spec No:179-94.

33. Dalle-Donne I, Carini M, Orioli M, Vistoli G, Regazzoni L, Colombo G, et al. Protein carbonylation: 2,4-dinitrophenylhydrazine reacts with both aldehydes/ketones and sulfenic acids. *Free Radic Biol Med* 2009;46(10):1411-9.

34. Damani LA, Nnane IP. The assessment of flavin-containing monooxygenase activity in intact animals. *Drug Metabol Drug Interact* 1996;13(1):1-28.

35. Kroemer G, Galluzzi L, Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 2007;87(1):99-163.

36. Yin MC, Hwang SW, Chan KC. Nonenzymatic antioxidant activity of four organosulfur compounds derived from garlic. *J Agric Food Chem* 2002;50(21):6143-7.

37. Pedraza-Chaverri J, Medina-Campos ON, Avila-Lombardo R, Berenice Zuniga-Bustos A, Orozco-Ibarra M. Reactive oxygen species scavenging capacity of different cooked garlic preparations. *Life Sci* 2006;78(7):761-70.

38. Garcia A, Haza AI, Arranz N, Delgado ME, Rafter J, Morales P. Organosulfur compounds alone or in combination with vitamin C protect towards N-nitrosopiperidine- and N-nitrosodibutylamine-induced oxidative DNA damage in HepG2 cells. *Chem Biol Interact* 2008;173(1):9-18.

39. Flora SJ. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxid Med Cell Longev* 2009;2(4):191-206.